AD NUMBER

AD857266

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; JUN 1969. Other requests shall be referred to Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.

AUTHORITY

Army Biological Defense Research Lab ltr dtd 29 Sep 1971

TECHNICAL MANUSCRIPT 502

DIFFERENCES IN THE THERMAL STABILITY AND LIPID COMPOSITION OF VEE VIRUS PROPAGATED IN DIFFERENT CELL CULTURE SYSTEMS

Fred P. Heydrick
Jo Ann F. Comer
Ralph F. Wachter

JUNE 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

12

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 502

DIFFERENCES IN THE THERMAL STABILITY AND LIPID COMPOSITION OF VEE VIRUS PROPAGATED IN DIFFERENT CELL CULTURE SYSTEMS

Fred P. Heydrick

Jo Ann F. Comer

Ralph F. Wachter

Virus & Rickettsia Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B061102B71A June 1969

ABSTRACT

Previous reports from this laboratory have indicated that certain biological and physical characteristics of Venezuelan equine encephalitis (VEE) virus are influenced by the host. Data from thermal stability experiments with partially purified and purified VEE virus suggest that virus propagated in L cell monolayers possesses greater thermal stability than similar suspensions of virus derived from chick fibroblast (CF) monolayers. The addition of 0.1% bovine serum albumin did not eliminate this stability difference. Lipid analyses of VEE virus propagated in these cell culture systems indicate that differences exist in the relative concentrations of the following phospholipid components: phosphatidyl choline, phosphatidyl ethanolamine, and sphingomyelin. Correlation between the difference in viral stability and alterations in the lipid composition of the virus has not yet been demonstrated.

I. INTRODUCTION*

It has been generally established that lipid-containing viruses acquire their outer envelope from host-cell lipid.[1,2] A comparison of the viral lipids of Rauscher virus isolated from the plasma of viremic mice and from an established cell line indicated that the plasma virus had more polyunsaturated fatty acids but the tissue culture virus contained more shorter chain saturated fatty acids.[3] Simpson and Hauser[4] reported that changes in the lipid content of myxoviruses brought about changes in biological activity; myxoviruses grown in chick fibroblast (CF) cells were highly susceptible to phospholipase C, whereas the same viruses propagated in the embryonated egg or in sphingomyelin-treated CF cells were resistant to the enzyme. Blough and Tiffany[5] reported that myxoviruses propagated in the presence of branched-chain fatty acids showed incorporation of this type of fatty acid and alteration of the relative proportion of acyl groups in the viral lipid. Influenza virus treated in this way showed increased pleomorphism and increased hemolytic activity toward chick red blood cells.

The purpose of this paper is to report that differences have been observed in the thermal stability of Venezuelan equine encephalitis (VEE) virus propagated in CF and L cell cultures, as well as differences in the lipid composition of virus from the two host systems. That correlation might exist between the thermal stability of VEE virus and the relative proportions of different types of viral lipid components is being investigated.

II. MATERIALS AND METHODS

Partially purified suspensions of the Trinidad strain of VEE virus that had been passed from the chick embryo host 10 times in CF cell monolayers or in L cell monolayers were employed in the stability experiments. Virus was partially purified from cell culture fluids by centrifuging in a No. 30 Spinco rotor at 11,700 x g for 15 minutes, followed by 73,350 x g for 1.5 to 3 hours. Sediments of the high-speed centrifugation were rinsed twice with buffer, then resuspended to original volume in 0.02 M phosphate buffer, pH 7.8, and distributed to plastic tubes with tight-fitting plastic caps. Optical density measurements at 260 and 280 mμ were employed to assure that the protein contents of the suspensions being compared were approximately the same.

---

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

Highly purified virus was prepared for stability studies and lipid analysis by layering partially purified virus onto a linear sucrose gradient and centrifuging in a Spinco SW-25 swinging bucket rotor at 53,500 x g for 3 to 3.5 hours. The band of purified virus was collected by puncturing the side of the tube.

Purified virus preparations labeled with P^{32} were extracted for lipid with chloroform-methanol (2:1) at 50 C in an atmosphere of nitrogen for two 15-minute periods. Labeled viral lipid was subjected to mild alkaline and acid hydrolysis, the products of which were separated by two-dimensional paper chromatography. Radioautographic methods were used to locate the labeled compounds and the radioactivity was quantitated by scintillation spectroscopy.

III. RESULTS

A. THERMAL STABILITY TESTS

A difference in the stability of the CF cell virus and the L cell virus, observed in tests at 4 C, is shown in Table 1. In all stability tests, viral infectivity was assayed in 14-day embryonated eggs or L cell monolayers. Results are expressed as either egg LD_{50} per milligram protein or plaque-forming units (pfu) per milligram protein. For VEE virus derived from CF monolayers, the rate of infectivity loss was more rapid than for virus propagated in L cells. As indicated by the data, both partially purified and purified L cell virus preparations possessed 100 times more infectivity at the time of final sampling than corresponding CF cell virus. Since purified virus preparations were stored as 30% sucrose suspensions during these tests, stabilization of infectivity by the sucrose extended the sampling period to 63 days, at which time the 100-fold difference in infectivity between virus propagated in the two hosts was observed.

A comparison of the stability at 25 C of partially purified virus from the two host systems is shown in Table 2. Triplicate samples were assayed daily for 5 days. Virus derived from the CF cell lost all infectivity in 5 days; in contrast, virus derived from the L cell retained more than 6 logs of infectivity at this time.

To determine whether the difference in thermal stability would still be manifest in the presence of stabilizing protein, bovine serum albumin (BSA) was added to purified and partially purified virus suspensions derived from both host cells. Partially purified virus was resuspended in either 0.02 M phosphate buffer, pH 7.8, or in buffer containing 0.1% BSA.

TABLE 1. COMPARISON OF STABILITY AT 4 C OF VEE VIRUS PROPAGATED IN CF CELL AND L CELL MONOLAYERS

Host	Specific Infectivity (pfu/mg protein) x 10^7[a/] at Indicated Days at 4 C					
	0	14	28	35	42	63
			Partially Purified VEE Virus			
CF cell	200	50	4.7	0.11	0.01	ND[b/]
L cell	110	62	35	6.2	2.2	ND
			Purified VEE Virus			
CF cell	1,600	540	100	47	ND	0.31
L cell	1,000	600	210	130	ND	23

a. Average of triplicate samples.
b. Not done.

TABLE 2. COMPARISON OF STABILITY AT 25 C OF PARTIALLY PURIFIED VEE VIRUS DERIVED FROM CF CELL AND L CELL MONOLAYERS

Host	Specific Infectivity (pfu/mg protein) x 10^6[a/] at Indicated Days at 25 C					
	0	1	2	3	4	5
CF cell	2,300	440	4.7	1.3	0.002	—[b/]
L cell	3,600	2,100	350	330	23	8.9

a. Average of triplicate samples.
b. Not detectable.

Data presented in Table 3 were obtained in two separate experiments in which BSA was added to partially purified VEE virus suspensions derived from the two host systems. In the presence of BSA the stability of virus from both hosts was enhanced, but a greater stability of the L cell virus was observed. In the first test (data in upper half of table), a significant difference was noted at 28 days; in the second test, a much greater difference in the stability of the CF and L cell virus was seen throughout the test period.

TABLE 3. EFFECT OF BOVINE SERUM ALBUMIN ON STABILITY AT 25 C OF PARTIALLY PURIFIED VEE VIRUS PROPAGATED IN CF CELL AND L CELL MONOLAYERS

Host	Specific Activity x 10⁶[a] at Indicated Days at 25 C				
	0	7	14	21	28
			Pfu/mg Protein[b]		
CF cell	300	5.5	1.0	0.16	0.0002
L cell	370	4.7	1.4	0.25	0.006
			Egg LD_{50}/mg Protein[b]		
CF cell	1,000	1	0.1	0.002	0.0001
L cell	250	2	1.0	0.2	0.1

a. Samples contained 0.1% bovine serum albumin.
b. Average of triplicate samples.

In Table 4 data are presented that compare the relative stabilities at 25 C of 30% sucrose suspensions of purified VEE virus derived from the two host systems. Data for samples without BSA indicate that after 4 days at 25 C, virus from the L cell host retained ten times more infectivity than the CF cell virus. The addition of 0.1% albumin to the purified virus suspensions extended the testing period to 35 days. After an initial sharp decrease in infectivity, both types of virus lost infectivity at the same rate until day 21. From this time until the final sampling at day 35 a more rapid rate of infectivity loss was shown by the CF-propagated virus.

TABLE 4. COMPARISON OF STABILITY AT 25 C OF PURIFIED VEE VIRUS PROPAGATED IN CF CELL AND IN L CELL MONOLAYERS

Days at 25 C	Specific Infectivity (pfu/mg protein) x 10[a/]			
	No BSA		0.1% BSA	
	CF Cell	L Cell	CF Cell	L Cell
0	2,300	4,000	230	320
1	820	1,800		
2	140	260		
3	72	270		
4	1.0	11		
7			5.2	10
14			1.8	3.2
21			0.32	1.2
28			0.004	0.030
35			0.0002	0.0034

a. Average of triplicate samples.

B. PHOSPHOLIPID ANALYSIS

VEE virus purified from L cell and CF cell hosts was found to contain approximately 22% lipid. However, VEE virus phospholipid, which for Sindbis virus was reported to be approximately 75% of the total lipid,[2] appeared to differ in composition depending upon the host cell used for propagation. These differences in viral phospholipid composition were based on the distribution of P^{32} in the deacylated glycerophosphate esters (Table 5). The lipid from virus of L cell origin contained the most radioactivity in the glycerophosphoroethanolamine derivatives (diacyl form plus plasmalogen form), while the lipid from virus of CF cell origin contained the most radioactivity in the glycerophosphorocholine derivative. In addition, sphingomyelin represented more than 20% of the total phospholipid of the L cell virus, while the CF cell viral lipid contained only half the activity of this component.

Analyses of L cell and CF cell viral phospholipids, separated by the two-dimensional thin-layer chromatographic method of Rouser,[6] indicated that spots from silica gel G plates corresponding to phosphatidyl ethanolamine, phosphatidyl choline, and sphingomyelin contained percentages of radioactivity similar to those of the deacylated lipids. Phosphorus analysis of these compounds separated by thin-layer chromatography was performed using the method of Bartlett.[7] The results confirmed the P^{32} distribution in that the L cell virus contained more lipid phosphorus as phosphatidyl ethanolamine and less as phosphatidyl choline while the CF cell virus had more lipid phosphorus present as phosphatidyl choline and less as phosphatidyl ethanolamine.

TABLE 5. GLYCEROPHOSPHATE ESTER COMPOSITION OF VEE VIRUS PURIFIED FROM CHICK FIBROBLAST CELLS AND FROM L CELLS

Component[a]	Composition, Per Cent[b]	
	CF Cell Virus	L Cell Virus
Unknown	0.23	0.13
GP Inositol	1.58	0.26
GPGP Glycerol	0.47	0.21
GP Serine	17.[illegible]7	17.00
Glycerol Phosphate	0.98	1.29
GP Glycerol	0.40	0.08
Cyclic Glycerol	0.24	0.48
GP Ethanolamine	7.18	13.40
GP Ethanolamine-P[c]	21.76	21.19
GP Choline	35.98	21.26
Unknown	0.83	1.37
Sphingomyelin	12.84	23.18

a. Prefix GP = glycerophosphoro-.
b. Based on distribution of P^{32}.
c. Plasmalogen.

IV. DISCUSSION

VEE virus propagated in the L cell host exhibits greater thermal stability at a variety of temperatures than does virus propagated in the CF cell host. BSA did not overcome what appears to be the basically less stable nature of CF cell VEE virus, although albumin does increase the stability of both L cell and CF cell virus. Other workers[4,5] have shown that alterations in the lipid composition of myxoviruses produce changes in biological characteristics. Although correlation between the difference in viral stability and alterations in the lipid composition of VEE virus has not yet been demonstrated, thermal inactivation may begin with a molecular degradation of the viral lipoprotein coat, and the differences in the content of the structural phospholipids may play a role in controlling these changes.

LITERATURE CITED

1. Kates, M.; Allison, A.C.; Tyrell, D.A.; James, A.T. 1962. Origin of viral lipids. Cold Spring Harbor Symp. Quant. Biol. 27:293-301.

2. Pfefferkorn, E.R.; Hunter, H.S. 1963. Purification and partial chemical analysis of Sindbis virus. Virology 20:433-445.

3. Johnson, M.; Mora, P.T. 1967. Lipids of Rauscher mouse leukemia virus. Virology 31:230-237.

4. Simpson, R.W.; Hauser, R.E. 1966. Influence of lipids on the viral phenotype. Virology 30:684-697.

5. Blough, H.A.; Tiffany, J.M. 1969. Incorporation of branched-chain fatty-acids into myxoviruses. Proc. Nat. Acad. Sci. 62:242-247.

6. Rouser, G.; Kritchevsky, G.; Galli, C.; Heller, D. 1965. Determination of polar lipids: Quantitative column and thin-layer chromatography. J. Amer. Oil Chemists' Soc. 42:215-227.

7. Bartlett, P. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234:466-468.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
DIFFERENCES IN THE THERMAL STABILITY AND LIPID COMPOSITION OF VEE VIRUS PROPAGATED IN DIFFERENT CELL CULTURE SYSTEMS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
Fred P. Heydrick
Jo Ann F. Comer
Ralph F. Wachter

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
June 1969	13	7

8a. CONTRACT OR GRANT NO.

b. PROJECT NO 1B061102B71A

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
Technical Manuscript 502

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT
Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

Previous reports from this laboratory have indicated that certain biological and physical characteristics of Venezuelan equine encephalitis (VEE) virus are influenced by the host. Data from thermal stability experiments with partially purified VEE virus suggest that virus propagated in L cell monolayers possesses greater thermal stability than similar suspensions of virus derived from chick fibroblast (CF) monolayers. The addition of 0.1% bovine serum albumin did not eliminate this stability difference. Lipid analyses of VEE virus propagated in these cell culture systems indicate that differences exist in the relative concentrations of the following phospholipid components: phosphatidyl choline, phosphatidyl ethanolamine, and sphingomyelin. Correlation between the difference in viral stability and alterations in the lipid composition of the virus has not yet been demonstrated.

14. Key Words
*Venezuelan equine encephalitis virus
*Temperature
*Stability
Lipids
Cells (biology)
Cultures (biology)
L cells
Infectivity

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification